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HISTOCHEMICAL CHANGES IN GONADAL NUTRIENT RESERVES CORRELATED WITH NUTRITION IN THE SEA STARS, *PISASTER OCHRACEUS* AND *PATIRIA MINIATA* [1]

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The annual reproductive cycle of a population of *Pisaster ochraceus* near Monterey, California, for the years 1954 through 1958 has been reported by Feder (1956), Greenfield (1959), and Farmanfarmaian, Giese, Boolootian, and Bennett (1958). Mauzey (1966) has reported the cycle for *Pisaster* at San Juan Island off the coast of Washington for 1962 and 1963. Comparable information is available for *Patiria miniata* for 1955 (Farmanfarmaian *et al.*, 1958) and from mid-1962 through early 1964 (Lawrence, 1965).

In addition to general delineation of the reproductive cycles for these two species, interest has been focused on biochemical changes occurring in rhythm with the cycles. Greenfield, Giese, Farmanfarmaian, and Boolootian (1958) have reported on lipid, protein, nonprotein nitrogen, and glycogen in the gonads and other organs of *Pisaster*, while Giese (1966a, b) gives more detailed information for both *Pisaster* and *Patiria*. Allen and Giese (1966) have investigated lipogenic rates in the gonads of *Pisaster*. Nimitz (1971) has reported histochemically detectable changes in the nutrient reserves of the gut of *Pisaster* and *Patiria*, as correlated with the reproduction and nutrition.

The present paper describes the localization of certain classes of fats and carbohydrates in the gonads of *Pisaster ochraceus* and *Patiria miniata* during the course of the reproductive cycle and correlates information gained through these histochemical studies with biochemical findings.

Materials and Methods

Nimitz (1971) describes in detail the sources and processing procedures for the specimens of *Pisaster ochraceus* and *Patiria miniata* on which the studies reported here are based. Samples of gonads taken from two males and two females each month for two years were fixed, sectioned and stained with techniques for various classes of carbohydrate and lipid and occasionally for protein.

The relative abundance of oocytes in different stages of development at different times of year (Fig. 1) was determined by classifying 50 oocytes per specimen into one of the three size classes. The relative abundance of spermatocytes was determined by measuring the height of the columns of spermatocytes extending into the lumen. Sperm were noted as absent or present in small, moderate or large quantities.

Results

Cyclic changes in the gonads

Pisaster and *Patiria* have two gonads in each arm and each empties separately by a short gonoduct at a pore in the angle between the arms. Both ovaries and

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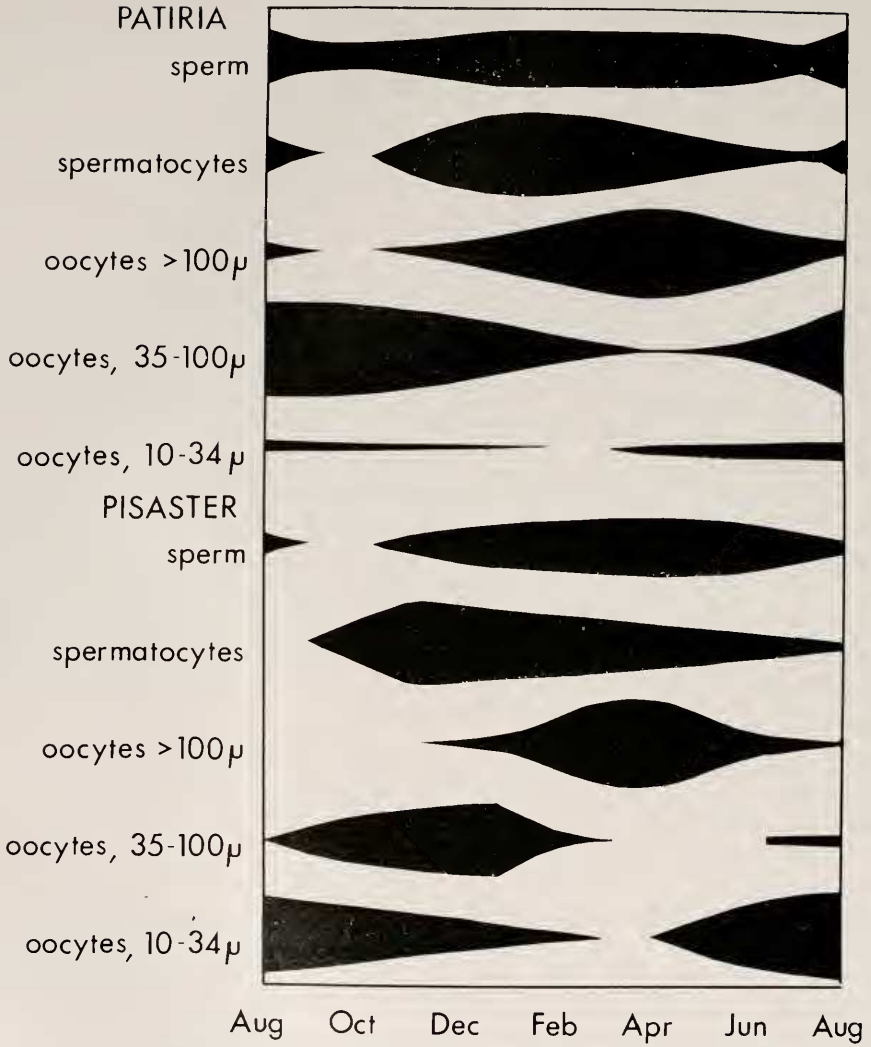

FIGURE 1. Seasonal abundance of germinal cells and sperm in the gonads of *Pisaster ochraceus* and *Patiria miniata*. The principle spawning period is indicated by the decrease in number of oocytes greater than 100 μ in diameter.

testes vary markedly in size in the course of the reproductive cycle in *Pisaster*. The gonads of *Pisaster* are smallest in August and September (GI = < 1), begin to enlarge about October, and reach a maximum size (GI = 16 or more) in March, April or May. Spawning occurred in May 1965 and between March and June 1966 (Nimitz, 1971, Fig. 7). Early in gametogenesis the testis and ovary are indistinguishable unless examined microscopically. Ripe ovaries are salmon pink; ripe testes, a cream color.

The gonadal cycle for *Patiria* is less pronounced and also less consistent. The gonads begin to increase in size in early fall. Over the period studied spawning

occurred in July 1965 and in May of both 1966 and 1967 (Nimitz, 1971, Fig. 8). The lesser degree of synchronization of gametogenesis and spawning in *Patiria* is reflected in the smaller overall range within which the average monthly gonad indices fall (1.5 to 9.6) as compared with the range in *Pisaster* (0.5 to 19.7). Individual specimens of *Patiria* reach gonad indices ranging from 0.5 to 15.4, but these extremes do not show up in the monthly averages because of the presence of animals in any given sample tending to opposite extremes. The ripe ovaries of *Patiria* are a brownish-orange; the ripe testes, a cream color.

The histology and histochemistry of the gonads of freshly-collected specimens

The following layers of the gonadal wall, distinguished in the combined light and electron microscopy studies by Davis (1971) and by Walker (1974), were likewise distinguished in the present study: the cuboidal peritoneal cells, the connective tissue layer, and the outer layer of muscle fibers of the outer sac; the perihaemal sinus (genital coelomic sinus); and the inner layer of muscle fibers, the haemal sinus and its walls, and the germinal epithelium of the inner sac. Other layers seen by electron microscopists could not be distinguished by the present investigator.

In a fully ripe testis from *Pisaster* with sperm filling the lumen (Fig. 2), there are along the connective tissue layer of the inner sac numerous oval cells 7 to 8 μ in length. The round to oval granular nucleus of each cell is 5 to 6 μ in diameter or length and contains a prominent nucleolus. These cells are interpreted as spermatogonia on the basis of the earlier work of Delavault and Bruslé (1968) on *Asterina gibbosa*. The lumen of the ripe testis is filled with sperm with spherical heads approximately 1.4 μ in diameter. At this stage, and in the succeeding one after the sperm have been released, there are often seen irregular yellowish granules in the peritoneal cells.

Ripe testes and recently-spawned testes also contain somewhat irregularly shaped cells approximately 10 μ in diameter with oval nuclei (2×3 μ) with a few basophilic granules. The cytoplasm of these cells is filled with yellow granules or globules, 0.5 μ in diameter (Fig. 3). These granules or globules stain with Sudan black B in both frozen and paraffin sections but were negative to all other stains utilized. Evidence which would permit identification of these cells either with the interstitial cells of *Asterina* testis (Delavault and Bruslé, 1968) or nutritive phagocytes of sea urchins (Holland and Giese, 1965) is lacking, though it seems unlikely that they are the same. They are distinctly different from cells of the vesicular tissue of *Asterina* in predominantly ovogenetic activity (Bruslé Tereygeol and Delavault, 1970).

In a recently spawned testis the spermatogonia and other smaller cells are crowded together in irregular clusters along the edge of the testis because of the contraction of the wall after the release of the mass of sperm. As the spermatogonia divide, columns of cells presumed to be primary spermatocytes extend into the lumen of the testis (Fig. 4). The nuclei of the primary spermatocytes are approximately 2.5 μ in diameter and stain rather darkly, although a faint granulation can be distinguished. The spermatocyte columns lengthen, and later in the season spermatids with nuclei 1.4 μ across occur at the inner tips of the columns. Secondary spermatocytes are probably not seen because they divide rapidly to form

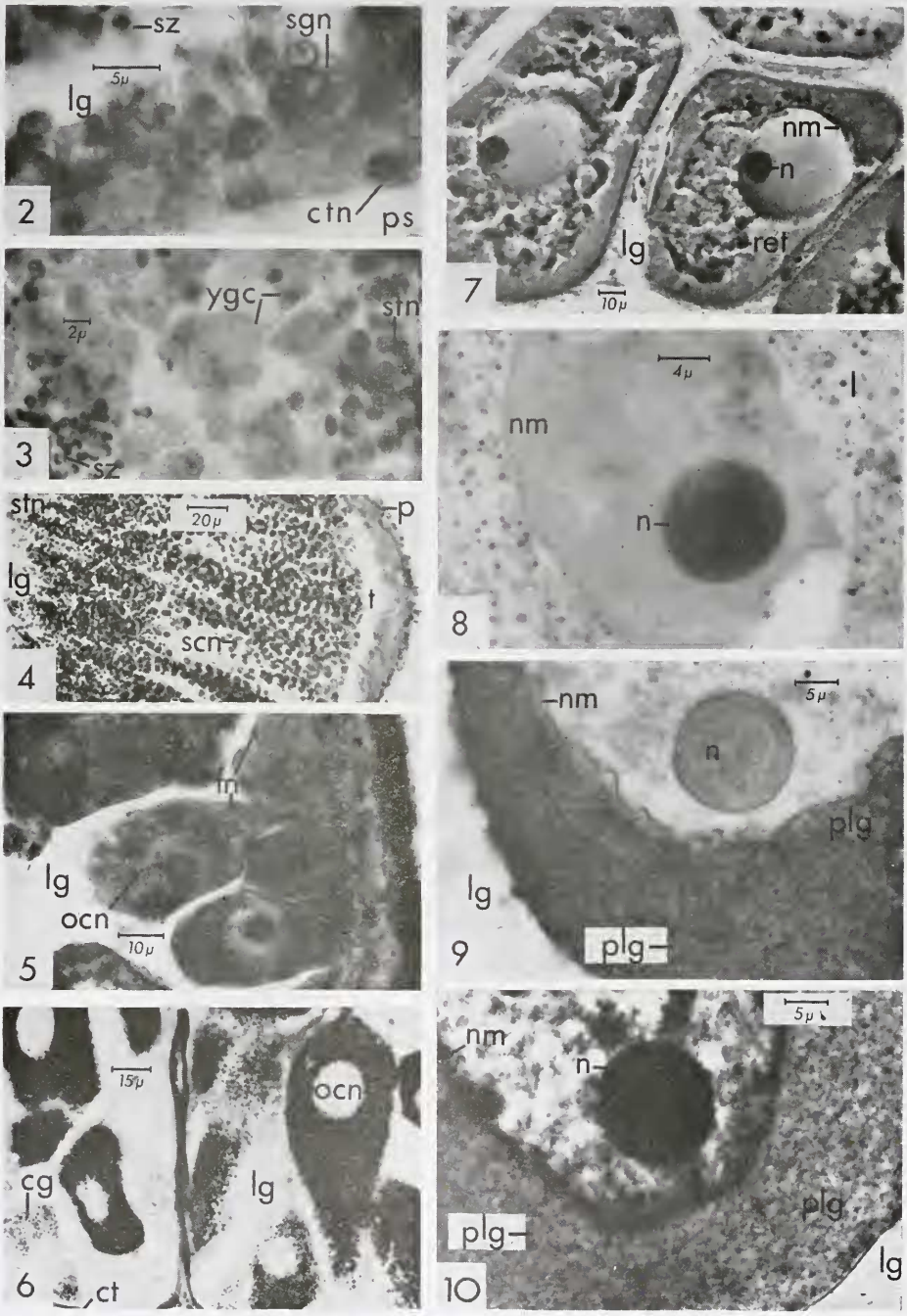

FIGURE 2. Section from inner wall of ripe testis of *Pisaster ochraceus*. stained with Prenant's triple stain showing spermatogonia. Symbols used are: ctn, connective tissue cell nucleus;

the spermatids. Spermatids metamorphose into sperm which dissociate from the columns and become evenly distributed throughout the lumen. Spermatogenesis in *Patiria* is essentially similar to the process in *Pisaster*.

The relative abundance of several types of germinal cells during the annual cycle is shown in Figure 1. In both species studied, developing columns of germinal cells without sperm in the lumen were seldom seen, suggesting that the span of time between the formation of primary spermatocytes and the maturation of sperm in any individual animal is relatively short. In Figure 1 the principal spawning period for both species corresponds to the period when the number of oocytes greater than 100 μ in diameter is decreasing.

Many of the histochemical results (Table I) were the same for the testes of both species and they will be described together here. Glycogen or a glycogen-like carbohydrate was present in the cytoplasm of the spermatocytes and to a lesser extent in the spermatids. There is no evidence for glycogen-like carbohydrate in the sperm; small amounts may be present in the spermatogonia. The peritoneal cells also contain granules of glycogen-like carbohydrate early in gametogenesis. In later stages the peritoneal cells are much flattened through stretching as the testis fills with sperm, and the presence of carbohydrate cannot be detected.

Most testicular tissue is consistently negative for all tests for neutral lipid. Irregular yellow granules seen in the peritoneal cells and yellow granules present in the "yellow granule- or globule-containing cells" in the lumen of the testis stain with Sudan black B in frozen sections and in Elftman's technique for phospholipid (though not with hematoxylin at pH 3) suggesting a bound lipid component.

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ps, perihaemal sinus (genital coelomic sinus); lg, lumen of gonad; sgn, nucleus of spermatogonium; sz, spermatozoa.

FIGURE 3. Edge of lumen of ripe testis of *Patiria,* stained with Harris's hematoxylin, showing the yellow granule- or globule-containing cells. Symbols used are: ygc, yellow granule-containing cells; stn, nuclei of spermatids; sz, spermatozoa.

FIGURE 4. Section from one lobe of testis of *Pisaster,* stained with Prenant's triple stain, showing the spermatogenic columns extending into the lumen. Symbols used are: lg, lumen of gonad; p, peritoneum; scn, nuclei of spermatocytes; stn, nuclei of spermatids; t, tear in tissue, not a sinus.

FIGURE 5. Section from wall region of one lobule of ovary of *Pisaster,* stained with Prenant's triple, showing the small oocytes which remain after spawning and the surrounding follicle cells. Symbols used are: fn, nuclei of follicle cells; lg, lumen of gonad; ocn, nucleus of oocyte.

FIGURE 6. Section from one lobe of ovary of *Pisaster,* sulfated and stained with methylene blue at pH 2.4, showing 0.5 to 1.5 μ granules of carbohydrate which accumulate during growth of the oocyte. Symbols used are: c g, carbohydrate granules, not removed by diastase; ct, connective tissue of ovary wall; lg, lumen of gonad; ocn, nucleus of oocyte.

FIGURE 7. Section of ripe oocytes of *Pisaster,* stained with Prenant's triple stain, showing coarse basophilic reticulum which has receded from the surface of the oocyte. Symbols used are: lg, lumen of gonad; n, nucleolus; nm, nuclear membrane; ret, coarse basophilic reticulum.

FIGURE 8. Section of oocyte of *Patiria,* nearly maximum size, stained with Harris's hematoxylin and oil red 0, showing accumulation of lipid globules in the cytoplasm. Symbols used are: n, nucleolus; nm, nuclear membrane; l, lipid globule.

FIGURE 9. Section of ripe oocyte of *Patiria,* stained with Sudan black B in Elftman's technique for phospholipid, showing phospholipid granules in the cytoplasm. Symbols used are: lg, lumen of gonad; n, nucleolus; nm, nuclear membrane; plg, phospholipid granules.

FIGURE 10. Section of ripe oocyte of *Patiria,* stained with hematoxylin at pH 3 in Elftman's technique for phospholipid. Symbols used are: n, nucleolus; nm, nuclear membrane; plg, phospholipid granules.

TABLE I

Histochemical results.

	Tests with positive staining
Gonad wall (both sexes)	
Peritoneal cell cytoplasm 0.2 μ granules or diffuse	Periodic acid-Schiff, glycogen sulfation, lithium silver (all three removed by diastase); occasionally standard sulfation
Connective tissue layers	Fast green, standard sulfation, alcian blue; periodic acid-Schiff, glycogen sulfation (both diastase resistant)
Muscle fibers Granules or diffuse	Periodic acid-Schiff (glycogen sulfation *Patiria* only), lithium silver (all three removed by diastase)
Perihaemal sinus coagulum	Periodic acid-Schiff (diastase resistant); gray-brown coloration with lithium silver; pale staining with Sudan III and IV and Sudan black B
Ovary	
Germinal epithelium and follicle cell cytoplasm 0.2 μ granules or diffuse	Periodic acid-Schiff, glycogen sulfation, lithium silver (all three removed by diastase)
Oocytes, 6–10 μ Fine basophilic granules	Harris' hematoxylin, azure A (blue), neutral red; turquoise with glycogen sulfation
Oocytes, 10–100 μ Basophilic reticulum, fine or coarse; basophilic islands or patches	Harris' hematoxylin, azure A (blue), neutral red; turquoise with glycogen sulfation
Granules (0.5–1.5 μ) uniformly scattered in pockets of fine reticulum, later confined to outer edge of cytoplasm or pockets in coarse reticulum	Periodic acid-Schiff, glycogen sulfation, lithium silver (all diastase resistant); standard sulfation
Granules (0.2 μ) mostly in pockets of the reticulum	Periodic acid-Schiff (removed by diastase)
Fine globules	Oil red 0, Sudan III and IV, Sudan black B
Granules (0.3 μ)	Elftman's hematoxylin and Sudan black B
Oocytes, 100–120 μ	
Coarse reticulum or patches	As under 10–100 μ oocytes
Four types of inclusions	As under 10–100 μ oocytes
Jelly layer	Standard sulfation, azure A B-metachromasia, alcian blue
Testis	
Spermatogonia cytoplasm	Pale periodic acid-Schiff and glycogen sulfation (both removed by diastase)
Spermatocytes and spermatids cytoplasm	Periodic acid-Schiff, glycogen sulfation, lithium silver, (all removed by diastase); standard sulfation occasionally; Elftman's hematoxylin and Sudan black B
Spermatozoa	Elftman's hematoxylin and Sudan black B
Cells with yellow granules or globules Yellow granules	Sudan black B (carbowax and paraffin sections)

Phospholipid is present in the developing gametes, but it is difficult to determine whether the positive staining with both parts of Elftman's technique is due to phospholipid in the membranes of cell organelles or to phospholipids stored in the cells.

A *Pisaster* ovary seen in late August (Fig. 5) is filled with growing oocytes, the largest of which are about 25 μ in diameter. The smallest recognizable germinal cells, probably oogonia, are approximately 5 μ across. Flattened follicle cells (Hamann, 1885; Bruslé and Delavault, 1968) surround small oocytes in some sections. The smallest recognizable oocytes, approximately 10 μ in diameter, have already accumulated some basophilic cytoplasmic granules, and these granules uniformly fill the cytoplasm of oocytes 25 μ across.

As they further enlarge the oocytes become somewhat tear-shaped because of their close association with the basement membrane. When the swollen end of the oocyte reaches a diameter of about 35 μ the basophilic material forms a fine reticulum in the meshes of which may be found 0.2 μ granules of a carbohydrate possibly removed by diastase (Table I). By the time the oocytes have reached a diameter of approximately 50 μ, yolk granules, each 0.5 to 1.5 μ in diameter, have begun to accumulate in the mesh (Fig. 6). A conspicuous component of these granules is PAS-positive carbohydrate not removed by diastase.

As the oocytes approach a diameter of 100 μ, the fine basophilic reticulum begins to recede towards the interior of the oocyte and becomes a coarse reticulum which then breaks up into isolated patches of darkly staining material (Fig. 7). The outer region of the cytoplasm is filled with the 0.5 to 1.5 μ carbohydrate yolk granules, and a few of these granules also occur in the pockets of the coarse basophilic mesh or between the patches of basophilic material.

In the pockets between strands of the coarse mesh there are also numerous smaller granules, 0.2 μ in diameter, which contain a carbohydrate which is perhaps removed by diastase and therefore glycogen; the judgment is a difficult one because of the number of intensely-staining carbohydrate yolk granules also present. The ripe oocyte is about 120 μ in diameter, contains a nucleus approximately 40 μ in diameter, and a nucleolus 12 to 14 μ in diameter.

Even in the ripe ovary small oocytes 10 μ in diameter are present along the wall, and as Mauzey (1966) also suggests, these oocytes will probably be left after spawning to mature in the succeeding breeding cycle. Once the ripe oocytes have been spawned, one occasionally sees in the lumen cells with yellow granules or globules similar to those in the testes. Irregular yellow granules similar to those seen in the peritoneum of the testes are also seen in the peritoneal cells of the ovary.

The development of the oocyte in *Patiria* is essentially as described in *Pisaster*.

In addition to the two types of carbohydrate granules mentioned above, the cytoplasm of the oocytes of *Patiria* and *Pisaster* also contains neutral lipid droplets (Fig. 8) and phospholipid granules (Figs. 9 and 10); there also seem to be granules unusually rich in protein, though the heavy background staining makes this conclusion only tentative. Whether the protein, carbohydrate and phospholipid occur in different granules or are combined or layered in one or two types of granules or platelets cannot be determined from available materials.

Generally in March or April (though sometimes as early as January) a clear extracellular layer appears around the fully grown oocytes of *Patiria* and *Pisaster*.

The staining affinities of this layer, which probably corresponds to the jelly layer of embryologists, are those of an acid mucopolysaccharide, possibly weakly sulfated.

Early in the breeding cycle the perihaemal sinus of the ovary and testis in both *Pisaster* and *Patiria* often contains carbohydrate substance not removed by diastase. In addition the material in the sinus occasionally stains diffusely with neutral lipid dyes.

Gonad index and histochemistry of gonads in starved animals

Starvation of specimens of *Pisaster* and *Patiria* is unquestionably associated with a reduction in the number of gametes produced, as reflected in the gonad index determinations (Nimitz, 1971, Table III). Specimens of *Pisaster* collected on December 15, 1964, and starved for fourteen months until February 7, 1966, had an average gonad index of 0.33 ± 0.25 (standard deviation) in contrast to field animals collected in February which had an average gonad index of 5.4 ± 3.3. Some of the starving animals had spawned fairly copiously in the tanks at the Hopkins Marine Station on August 15, 1965 (Giese, personal communication). The specimens of *Pisaster* starved for twenty months until July 27, 1966 had an average gonad index of 0.15 ± 0.02 as opposed to an index of 3.2 ± 3.02 for animals freshly collected from the field. In one sense, using July field animals which had spawned as controls, does not show the extent of the impact of twenty months of starvation as well as it might. The starving animals had presumably not spawned in 1966 and thus would be more appropriately compared to the March field animals which had not spawned and which had an average index of 15.1 ± 4.5. Unfortunately the "field animals" in the above study could not be taken from the same population from which the starving animals had come, which may cast some doubt on the validity of using them as controls (Nimitz, 1971).

In *Patiria* the starving animals presumably had an initial average gonad index of 2.8 ± 3.1 as determined from the gonad index determination of a sample taken from the field at the same time as the animals placed under starvation. The gonad index of the starving animals shrank to an average of 1.8 ± 1.3 in May after eight months of starvation, as compared to May field animals with an average index of 4.0 ± 1.7.

Only two testes of starved *Pisaster* were successfully processed. The testis of an animal starved fourteen months contained recognizable spermatogonia with a few granules of glycogen-like carbohydrate. The germinal epithelial cells and peritoneal cells of the specimen starved fourteen months and of the one starved twenty months contained moderate numbers of carbohydrate yolk granules. The specimen starved fourteen months showed neutral lipid globules in the germinal epithelial cells, and similar globules appeared in the peritoneal cells of the one specimen starved twenty months. Normal testes do not show neutral lipid droplets in either site. Numerous cells containing the yellow granules or globules were present in the lumen. These are possibly engaged in breaking down the germinal cells and/or gathering up the products of breakdown for transport and use elsewhere.

In the single ovary processed for carbohydrates from specimens starved for fourteen months, the largest oocytes were 24 μ in diameter and lacked the carbohydrate yolk granules. The peritoneal cells, germinal epithelium, and follicle

cells were moderately rich in 0.2 μ carbohydrate granules. Tissues from two specimens were processed successfully through lipid techniques, and some neutral lipid was present in the germinal epithelium and follicle cells.

Two ovaries from animals starved twenty months showed 0.2 μ granules of carbohydrate present in the germinal epithelial and peritoneal cells. In tissue processed for lipids there were found some oocytes 7 μ in diameter and 18 μ long with tiny lipid globules stainable with Sudan black B. Among the animals which had spawned after eight months of starvation and then starved an additional twelve months, no new cycle of gonadal growth had been initiated by the time of sacrifice (or if new germinal cells had been produced during the last year of starvation they had subsequently been resorbed).

The ovaries of *Patiria,* starved from late August to May, all showed a reduction in the number of large oocytes, and in three specimens, it appeared that the oocytes were being broken down. The large oocytes that remained contained fine droplets of neutral lipid, and in the areas of the ovary where resorption was occurring there were larger droplets of lipid in masses of debris near the ovary walls. Among the debris there appeared to be numerous isolated nucleoli, some still basophilic, others colorless, of various sizes. The oocytes stained strongly with carbohydrate techniques, but the staining appeared diffuse, not associated with discrete granules characteristic of normal oocytes. The other germinal epithelial cells (*i.e.*, would-be follicle cells and oogonia) and peritoneal cells stained strongly for carbohydrate, and the germinal epithelial cells may contain some neutral lipid. The positive staining for nutrient materials in the ovary is not surprising if the oocytes are being broken down. It is reasonable to expect that nutrients released during the breakdown would be taken up into the adjacent germinal epithelial cells.

The testes of starved *Patiria* contained fewer sperm than usual, but the normal spermatogenic cycle had apparently been carried to completion, since the lumen of each testis contained only ripe sperm; no columns of developing cells were seen. Spermatogonia were seen in moderate number in the germinal epithelium. The cells containing yellow granules or globules were unusually abundant in the lumen. There was no evidence of neutral lipid present in slides stained with oil red 0, but in Sudan black B-stained slides, the sperm appeared to contain tiny droplets of lipid in the thin layer of cytoplasm of the head region. This is possibly phospholipid, given the negative reaction with oil red 0. It is puzzling that lipid should show up as droplets in sperm of starved animals and not in normal ones, but this could have resulted from improved conditions for studying the sperm when they were not so crowded as usual. In three out of four specimens there was a diffuse staining in the testes for carbohydrates in both the germinal epithelial and peritoneal cells. All the carbohydrate staining, including that of connective tissue, was removed by pancreatin, but through human error no slides of starved *Patiria* gonad were run with amylase or diastase incubation.

Discussion

Farmanfarmaian *et al.* (1958) earlier reported spawning of *Pisaster ochraceus* in April 1954 and 1955 and March 1956, and Greenfield (1959) in June 1958. Farmanfarmaian *et al.* (1958) reported spawning of *Patiria* in June and July 1955 and Lawrence (1965) in June 1963 and January 1964.

Giese's (1966b) biochemical data indicate that the gonads in both sexes of specimens of *Pisaster* of low gonad index contain lipid amounting to about 5% of the dry weight. In the male there is an increase to 8% lipid (dry weight) in animals of intermediate gonad index, while animals with high index are poor in lipid ($3.5 \pm 5.2\%$). If one considers that about 5% of the lipid in tissue is structural lipid (Giese, 1966a), significant neutral lipid stores would not be expected in the testis. Allen (1964) indicates that 70% of testis lipid in *Pisaster ochraceus* is polar lipid, chiefly phospholipid. The histochemical findings of this present study support strongly both the scarcity of neutral lipid and the abundance of phospholipid.

The testes of *Patiria* contain roughly 18% lipid (Giese, 1966b). This lipid is not demonstrable as neutral lipid by the histochemical procedures used in the present study and is presumably phospholipid. The higher lipid content in the testes of *Patiria* than in testes of *Pisaster* may relate to the greater abundance in this species of the cells containing the yellow granules (globules?) which stain intensely with Sudan black B.

The ovary of *Pisaster* increases in per cent of dry weight of lipid from approximately 5 to 35% (Giese, 1966b) at the same time it is increasing in mass preparatory to breeding, and Allen (1964) has found that polar lipid, largely phospholipid, accounted for 70% of the lipid in immature gonads of *Pisaster* and for 55% of the lipid in ripe ovaries. The biochemically detected increase of lipid in the ovary of *Pisaster* correlates with the histochemical observations of neutral lipid droplets and phospholipid granules accumulating in great abundance in the oocytes.

The *Patiria* ovary averages about 20% lipid (Giese, 1966b). Work with other sea stars indicates total lipid varying with the reproductive cycle but always with high levels of polar lipid in the ovaries and low total lipid with high proportion of polar lipid in the testes (Pearse and Giese, 1966; Akino, Shimojo, and Sasaki, 1970; Lawrence, 1973).

Allen and Giese (1966) have found that the rate at which labeled precursors are incorporated into lipid in the gonads of *Pisaster ochraceus* increases from November to February. The increase in lipogenic rate in the gonads occurs about the time the hepatic caeca begin to shrink and probably at their expense.

A glycogen-like material accounts for 0.43% of the dry weight of the testis in *Pisaster* of low gonad index and this decreased to less than 0.1% in individuals of high gonad index. *Patiria* testis averages about 2% dry weight of glycogen-like material (Giese, 1966b). The carbohydrate is histochemically detectable in the cytoplasm of the spermatogonia and spermatocytes, though not in the sperm, in both *Pisaster* and *Patiria*.

The ovaries of *Pisaster* of low gonad index show a small amount of glycogen-like material in the germinal epithelial cells, peritoneum, and possibly also in the small oocytes, according to histochemical results. Such individuals were found by Giese (1966b) to have a glycogen-like carbohydrate content of 0.4%. During oogenesis the biochemically detectable glycogen-like carbohydrate is reduced to 0.25%. The *Patiria* ovary contains about 0.6% glycogen-like carbohydrate. The occurrence of histochemically detectable glycogen-like material in the larger oocytes is uncertain, because the existence of contrast between diastase or amylase-treated slides and the controls is difficult to judge. An abundance of carbohydrate resistant to diastase accumulates as 0.5–1.5 μ yolk granules in the oocytes during growth.

Mauzey (1966) has also found this carbohydrate refractory to amylase in his studies of *Pisaster,* and Chia (1968) found a similar refractory carbohydrate associated with protein and lipid in the yolk platelets of the oocytes of *Leptasterias hexactis.* The failure of the amylase or diastase to digest this carbohydrate does not rule out the possibility that it is glycogen, since glycogen complexed with protein might be protected from digestion.

J. M. Lawrence (unpublished data cited in Giese, 1966b) has found 15 to 20 mg of lipid per 100 g of body fluid in *Pisaster ochraceus,* 6.5 to 7.3 mg in *Patiria.* Glucose in the body fluid (A. L. Lawrence, unpublished data cited in Giese, 1966b) amounts to roughly 0.25 to 0.29 mg per 100 g in *Pisaster,* and 0.20 mg in *Patiria,* yet the total carbohydrate of the body fluid varies from 0.70 mg in *Pisaster* of low gonad index to 1.84 mg in specimens of of high gonad index. In *Patiria* the total carbohydrate ranged from 0.78 to 0.87 mg per 100 g of body fluid. Since glucose accounts for only a fraction of the total carbohydrate, other sugars and/or sugar polymers are probably present in the sea stars body fluids. The histochemical staining for carbohydrate in the perihaemal sinus of *Pisaster,* observed also by Mauzey (1966), probably represents carbohydrate polymers since simple sugars are assumed to wash out of tissue sections during histochemical processing.

The development of large amounts of vesicular tissue after spawning, as described for *Asterina gibbosa* by Bruslé, Tereygeol, and Delavault (1970), has no parallel in *Pisaster* and *Patiria.* This may correlate with the presence of ample storage space in the pyloric caeca, which do contain significant stores of lipid and amylase-refractory carbohydrate and which do, a least in *Pisaster,* show cyclic changes in size correlating with the reproductive cycle (Nimitz, 1971, Figs. 7 and 8). It seems probable that in *Pisaster* and *Patiria* nutrients used in gametogenesis are derived from reserves in the pyloric caeca.

Parallel to findings in this present study, Crump (1971) has shown a striking impact of food availability on reproductive potential in *Patiriella regularis,* since the average gonad index of animals fed crabs to satiety was 26.13 ± 10.69 as compared with the average gonad index of 1.36 ± 1.45 in animals starved for 44 weeks. (Crump defined GI as the ratio of the volume of the gonad to the body weight after removal of gonads and caeca, $\times 100$.) The gonads of *Patiriella* starved for 44 weeks likewise contained only immature gametes at a time when control animals showed gonads ready for spawning.

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Summary

1. *Pisaster ochraceus* has an annual reproductive cycle in which the gonads increase in size rapidly from October or November and reach a maximum in March to May, after which spawning occurs. In *Patiria miniata* the gonadal cycle is less

pronounced and less regular. In the course of this study, animals spawned in July 1965, May 1966, and May 1967.

2. Histochemical techniques indicate that in both species glycogen or a glycogen-like carbohydrate occurs in the germinal epithelium and the follicle cells of the female, and in the spermatogonia and primary spermatocytes. A storage carbohydrate which is not removed by diastase or amylase is abundant in oocytes in the form of yolk granules 0.5 to 1.5 μ in diameter.

3. Neutral lipid droplets and phospholipid granules are abundant in all oocytes but the smallest. In the testes, lipid droplets are seen only after prolonged starvation.

4. In both species prolonged starvation results in failure of the gonads to achieve their normal size increase. Such gametes as are seen in starving specimens appear histochemically normal in some instances; in other cases they seem to be undergoing breakdown.

5. The histochemical results concerning nutrient reserves of the gonads are generally in agreement with the biochemical findings of earlier workers.

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